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RESEARCH ARTICLE

Neuronal FasL Induces Cell Death of Encephalitogenic T Lymphocytes

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Apoptosis of inflammatory cells plays a crucial role in the recovery from autoimmune CNS disease. However, the underlying mechanisms of apoptosis induction are as yet ill-defined. Here we report on the neuronal expression of FasL and its potential function in inducing T-cell apoptosis. Using a combination of facial nerve axotomy and passive transfer encephalomyelitis, the fate of CD4$^+$ encephalitogenic T cells engineered to express the gene for green fluorescent protein was followed. FasL gene transcripts and FasL protein were detected in neurons by *in situ*-hybridization and immunohistochemistry. T cells infiltrating preferentially the injured brain parenchyma were found in the immediate vicinity of FasL expressing neurons and even inside their perikarya. In contrast to neurons, T cells rapidly underwent apoptosis. In co-cultures of hippocampal nerve cells and CD4$^+$ T lymphocytes, we confirmed expression of FasL in neurons and concomitant induction of T-cell death. Antibodies blocking neuronal FasL were shown to have a protective effect on T-cell survival. Thus, FasL expression by neurons in neuroinflammatory diseases may constitute a pivotal mechanism underlying apoptosis of encephalitogenic T cells.

Introduction

FasL (CD95L) and its surface receptor, Fas (CD95, Apo-1, FasR), are key molecules in the regulation of apoptotic pathways in the immune system. Fas-FasL interactions influence the life of T cells at various stages including during thymic development, peripheral T lymphocyte deletion, clonal expansion in response to antigenic stimuli, and normal aging (1, 31, 37, 43, 54, 55). Deficiency of FasL or its binding partner Fas results in uncontrolled lymphocyte growth leading to spontaneous autoimmune disorders (15, 27, 38). Expression of FasL is not restricted to lymphoid cells, but is constitutively present in a number of tissues many of which are known to be immune-privileged, including the nervous system (21, 45, 46, 50, 56). Whereas expression of FasL in the eye and the testis has been demonstrated to help maintain the immune privileged status of these organs, direct evidence for a similar role of FasL in the central nervous system (CNS) is still lacking (5, 24).

We now show that FasL is constitutively expressed on motoneurons of the brain stem and upregulated following the induction of neuronal stress in the axotomy model of the facial nerve. We further demonstrate that CNS antigen-specific, encephalitogenic T cells genetically modified to express the green fluorescent protein (GFP), preferentially infiltrate the lesioned facial nucleus. Here, T cells were observed in close contact with neuronal surface membranes and even found inside their perikarya. Morphological analysis and infiltration kinetics of the T cell response indicated rapid induction of apoptosis in these cells upon contact with neurons. *In vitro* experiments employing co-cultures of FasL-expressing hippocampal neurons and T cells confirmed the direct interaction of both cell types. Moreover, neutralizing anti-FasL antibody protected T cells against neuron-mediated killing.

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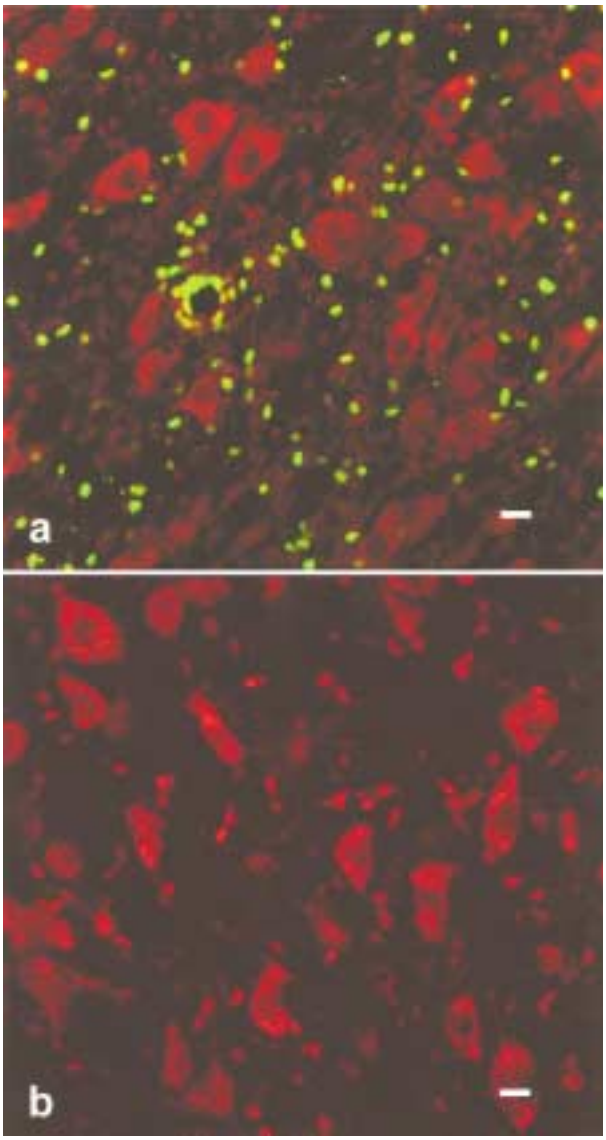


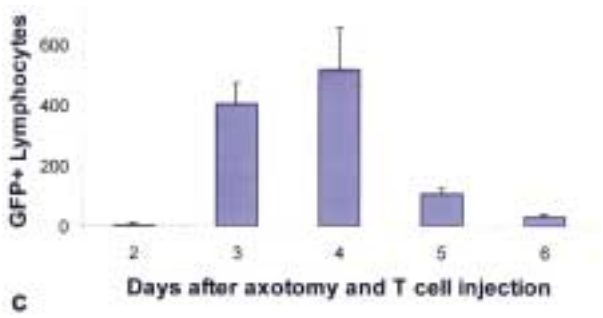
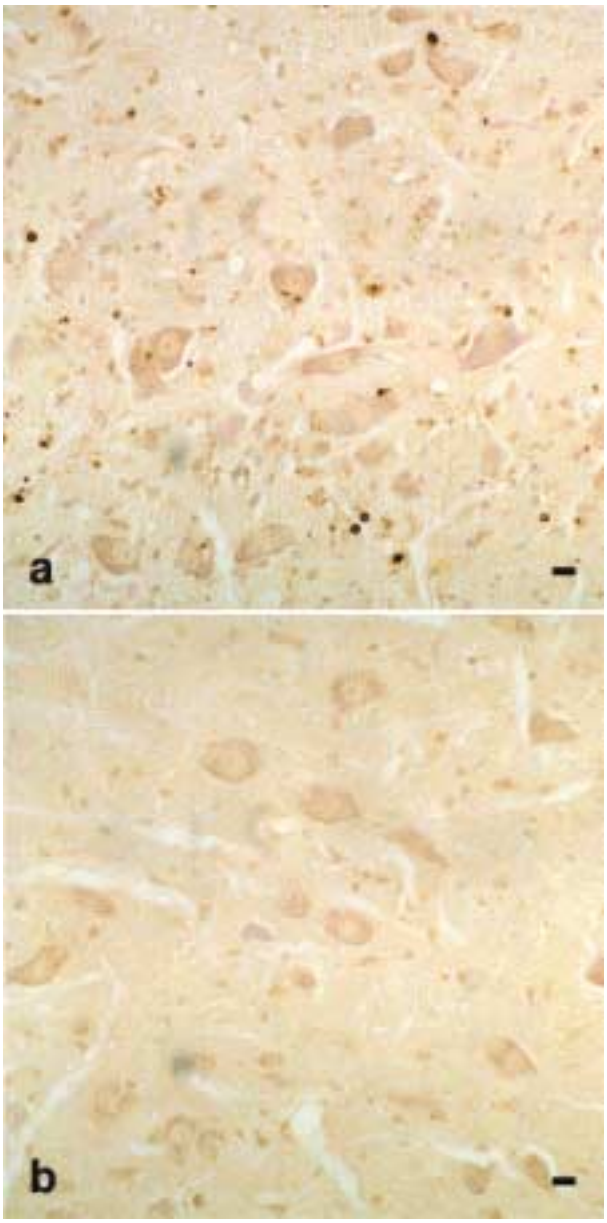


Figure 1. Post-traumatic invasion and death of CD4⁺ encephalitogenic T_{MBP} cells in regenerating CNS tissue. **a.** Transgenic T cells carrying the GFP marker gene (green) invade the axotomized facial nucleus 3 days following peripheral transection of the nerve as well as T cell transfer. Fluorescent red counterstaining with ToPro™. Confocal microscopy. Scale bar: 10 μm. **b.** In contrast, the contra-lateral non-axotomized nucleus of the same tissue section shows no T_{MBP}GFP cells. **c.** Kinetics of T cell invasion of the axotomized rat facial nucleus. Infiltration of GFP⁺ cells occurs very rapidly and reaches a maximum at day 4 after tEAE induction. Within a single day, 80% of the invading cells are cleared from the nucleus (day 5). Data were normalized for the number of motoneurons: [(Nt/Nn)*100], where Nt is the number of T cells and Nn is the number of neurons per nucleus and section (n=3).

Materials and Methods

T-cell lines. For the induction of transfer experimental autoimmune encephalomyelitis (tEAE), Myelin basic protein (MBP)-specific T lymphocytes were generated by subcutaneous immunization of Lewis rats with 150 μg guinea pig MBP emulsified in complete Freund's adjuvant containing 4 mg/ml Mycobacterium tuberculosis (Statens Seruminstitut, Copenhagen, Denmark). Inbred Lewis rats were obtained from the animal breeding facility of the Max-Planck-Institute of Biochemistry (Martinsried, Germany) and used at 6-8 weeks of age. MBP was purified from guinea pig brain as described (14). Ten days after hind pad injection, cells obtained from the draining popliteal lymph nodes were isolated and cultured following the limiting dilution protocol established by Pette and colleagues (42). In brief, 10^5 cells/well were cultured in the presence of 10 micrograms/ml MBP in 96-well plates for three days and propagated for another 6-8 days in IL-2 conditioned medium. For restimulation with antigen, T lymphocytes were co-cultured with 1×10^6 irradiated (5000 rad) syngeneic thymocytes/well as antigen presenting cells (APC's) and propagated as described (6). T lymphocyte antigen specificity was assayed in a standard radioactive proliferation assay.

Retroviral transduction of CD4⁺ T cells. The retroviral vector used for transduction of T-cells with the EGFP gene (Clontech Laboratories, Heidelberg, Germany) was constructed by cloning the cDNA (Clontech Laboratories, Heidelberg, Germany) into the BamHI restriction site of the retroviral vector, pLXSN (34). Packaging cell lines producing GFP-carrying retrovirus were established by introducing the pLGFPSN construct into the ecotropic packaging line GP+E 86 (33). Transduction of lymphocytes was performed as described elsewhere (16). In brief, GP+E 86 packaging cells producing replication deficient retrovirus (approx. 1×10^6cfu/ml) were seeded in 96-well plates (10^6/plate). Lymphocytes harvested from immunized animals were



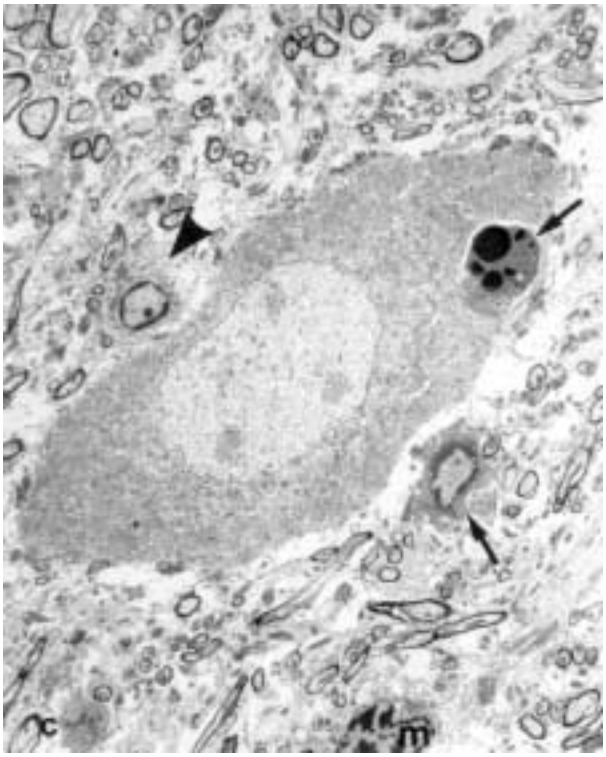


Figure 2. a, b. TUNEL labeling of apoptotic T cells (dark brown DAB reaction product) which are often located inside neurons as well as in their vicinity; lesioned (**a**) compared to the unlesioned (**b**) facial nerve nucleus 3 days after T-cell transfer. Hemalum counterstaining. Data are representative of three independent experiments using different animals and T-cell lines. Scale bar: 10 μm. **c.** Apoptotic T cell inside the perikaryon of a facial motor neuron 4 days after axotomy. Two GFP expressing lymphocytes recognizable by their electron dense, DAB positive cytoplasm (long arrows) and one GFP negative T cell (arrowhead) within and in the immediate vicinity of an axotomized facial motoneuron. The membrane of the neuronal nucleus shows ruffling characteristic of an axotomized cell and the stacks of rough endoplasmic reticulum cisternae normally present in healthy motor neurons are dispersed. Massive condensation and fragmentation of the T-cell nucleus inside the neuron indicates classical apoptosis. In contrast, no apoptosis of microglial cells (**m**) was observed. Transmission electron microscopy of immunocytochemically stained tissue using anti-GFP goat serum as the primary and peroxidase conjugated anti-goat serum as the detection antibody. Magnification: ×2,500.

added to the precoated wells (1×10^5/well) in the presence of antigen (10 micrograms/ml). After three days in culture, the T lymphocyte blasts were expanded in IL-2 containing growth medium. Selection with G418 (0.4 mg/ml) was started after three more days in culture in the presence of IL-2 conditioned medium and maintained during subsequent restimulations.

Axotomy model of the rat facial nerve. A total of 95 young adult Lewis rats was used for these experiments. Surgery was performed under deep ether anesthesia. The right facial nerve was cut close to its exit from the stylomastoid foramen. Injection of encephalitogenic GFP-transduced T lymphocyte blasts (5×10^6/animal) was performed intraperitoneally immediately after the operation. The animals were kept under standardized conditions at constant temperature (22°C), controlled lighting, and free access to food and water. For histological analysis, animals were perfused with 4%

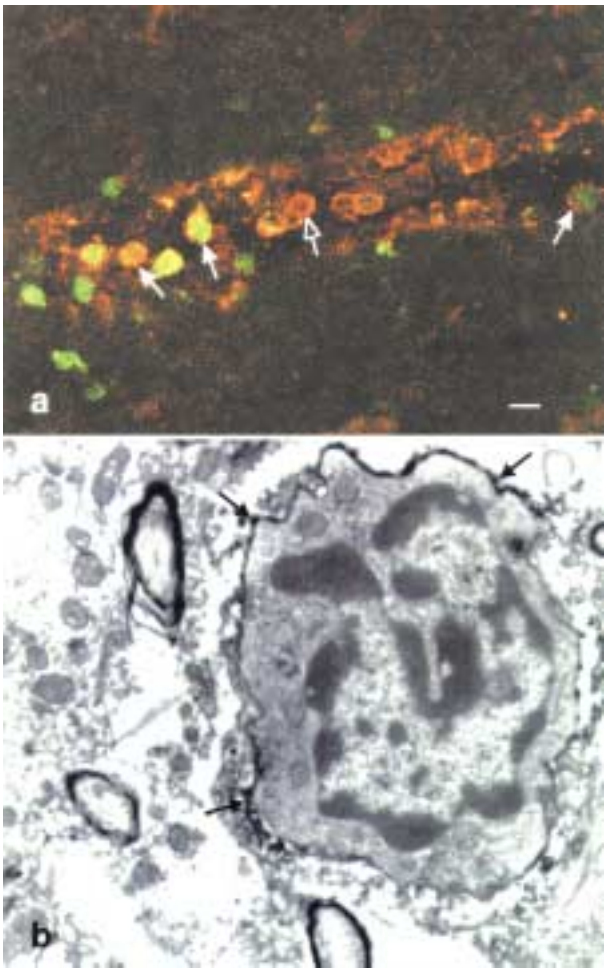


Figure 3. a. T_{MBP} GFP lymphocytes (green, closed arrows) and recruited cells (open arrow) invading the axotomized facial nerve nucleus (day 3 post T cell transfer and nerve transection) express Fas antigen on their surface membrane (Cy3 red fluorescent label). Confocal microscopy. Scale bar: 10 μm. **b.** Electron micrograph showing Fas immunoreactivity (peroxidase /DAB reaction product, arrows) on the surface of a T cell infiltrating the facial nucleus. Magnification: ×6000.

paraformaldehyde (PFA) in phosphate buffered saline (PBS). Postfixation of the brains was done in the same fixative at 4°C for 24 h followed by cryoprotection in 15% sucrose solution in PBS overnight. At least 4 animals were used for each of the different time points examined.

Immunohistochemistry and electron microscopy. For immunohistochemical staining, 20 μm cryostat sections were mounted on gelatinized glass slides and dried. Fixation for 30 min at RT in 4% PFA was followed by staining of the sections with polyclonal goat-anti-Fas and goat-anti-FasL antibodies (Transduction Laboratories, obtained from Dianova, Hamburg, Germany) for 1 hr at RT (1:125 dilution). Binding of primary antibodies was visualized using a biotinylated rabbit anti-goat serum preabsorbed with heat-inactivated normal serum from our Lewis strain 30 min before use. Avidin-biotin-peroxidase (Vectastain ABC-Kit, Vector Laboratories, Burlingame, CA, USA) served as the detection system with 3, 3′-diaminobenzidine (DAB) as an enzymatic substrate. In addition, detection of primary antibodies was performed using a rabbit anti-goat antiserum carrying Cy3 as the fluorescent tag (1:400 dilution). Immunofluorescence analysis was carried out using a Leica confocal laser microscope. Infiltrating GFP-positive cells were analysed after postfixation of 20 μm cryostat sections in 4% PFA. Counterstaining was performed with ToPro^{RM} (Molecular Probes, Leiden, Netherlands). For electron microscopy, animals were perfused as described above. Brains were postfixed for 24 h in PBS/4% PFA. Subsequently, sections of 60 μm thickness were cut using an Oxford vibratome. Staining with anti-Fas and polyclonal rabbit anti-GFP antibodies (1:100 dilution, Clontech Laboratories, Heidelberg, Germany, secondary biotinylated goat anti-rabbit antibody) was performed as described above except that all labelings were done on floating sections. The osmicated and dehydrated sections were finally embedded in Araldite and examined with a Zeiss EM10 transmission electron microscope. TUNEL staining was performed after the protocol of Gavrieli and colleagues (22).

RNA analyses. RNA was isolated from facial nerve nuclei as described (9) and treated with DNAse (Boehringer Mannheim, Mannheim, Germany). Purified mRNA was reverse-transcribed into cDNA using AMV reverse transcriptase (Boehringer Mannheim, Mannheim, Germany). FasL PCR amplification and cloning of *in situ* probes was done using the following primers, anti-sense, 5′-AAGCCAAAAAAGGTCTTAGATTCCTC-3′, and sense, 5′-CAGAACTCCGTGAGTTCACCAAC-3′, based on the protocol of Suda and colleagues (51). The resulting PCR product spanning 499 bp was subcloned in pZErO (Invitrogen, De Schelp, The Netherlands) and sequenced. *In situ* hybridization, histochemistry and the following washing steps were performed as described earlier (49). In brief, 0.2 ng of the cloned FasL vector was PCR amplified using primers specific for pZErO anchored with either the T7 or Sp6 RNA-polymerase recognition sequence and purified using the QiaQuick kit (Qiagen). Transcription with either T7 or Sp6 RNA-polymerase was carried out

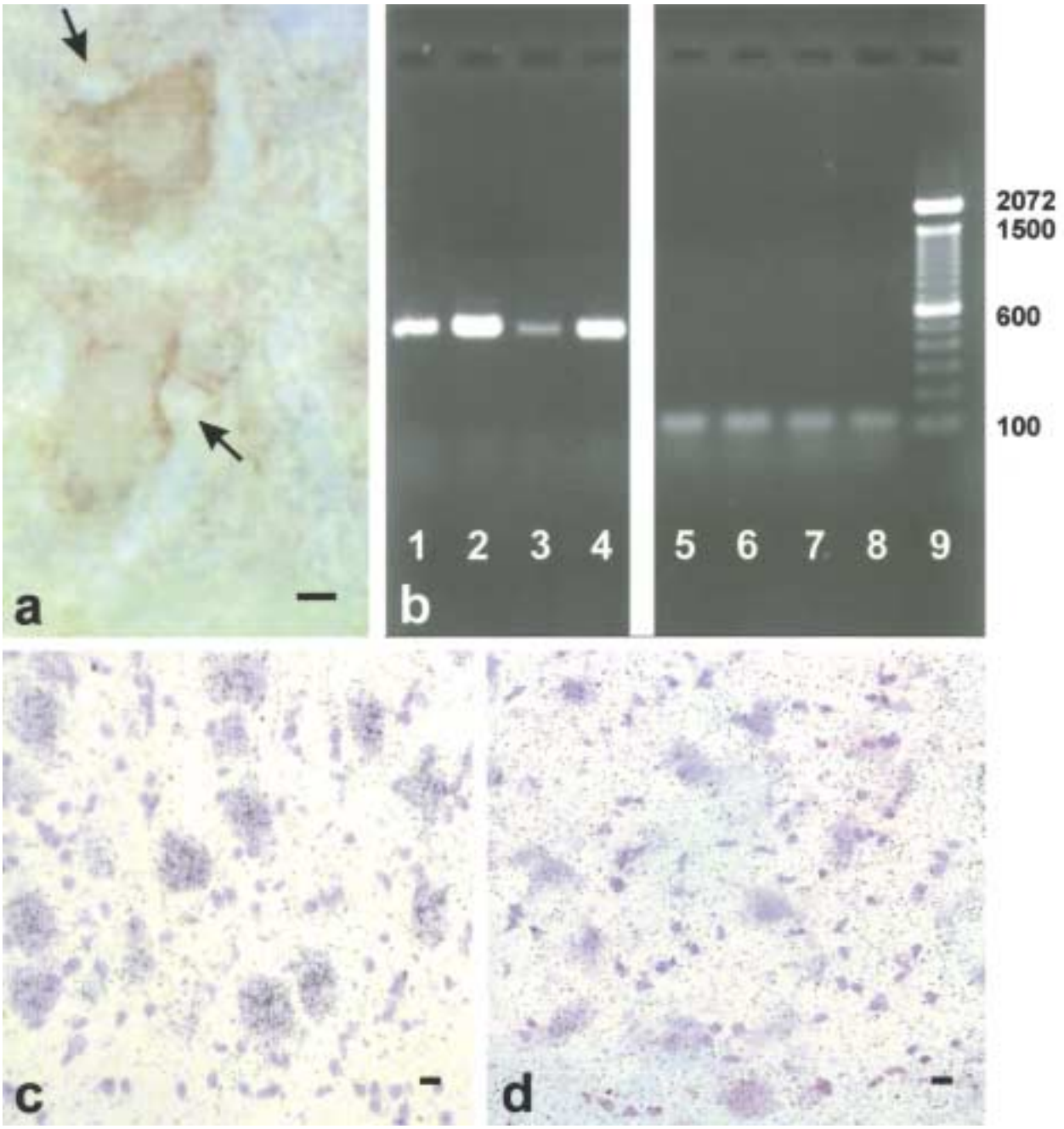


Figure 4. FasL expression by facial motoneurons. **a.** FasL immunoreactivity (peroxidase/DAB reaction product) is visible in areas of contact between the surface membrane of neurons and perineuronal cells (arrows). Four days post T-cell transfer and axotomy. **b.** RT-PCR demonstrates presence of FasL message both in the axotomized (lanes 1-2) and normal (lanes 3-4) rat facial nucleus (35 and 40 cycles, respectively). For control, template RNA was used to amplify the cyclophilin A gene (25 cycles; 5-6, operated nucleus; 7-8 contralateral unlesioned nucleus; cf. 24). Lane 9 shows a 100 bp DNA ladder. Facial nerve nuclei were prepared from rat brain stems four days after axotomy. **c.** *In situ* hybridization confirms expression of FasL by regenerating facial motoneurons. No specific hybidization signal is detectable in glial cells. Four days post facial nerve axotomy. **d.** *In situ* hybridization with the FasL-sense probe revealed no specific signal accumulation. Hemalum counterstaining. Scale bar: 10 μm.

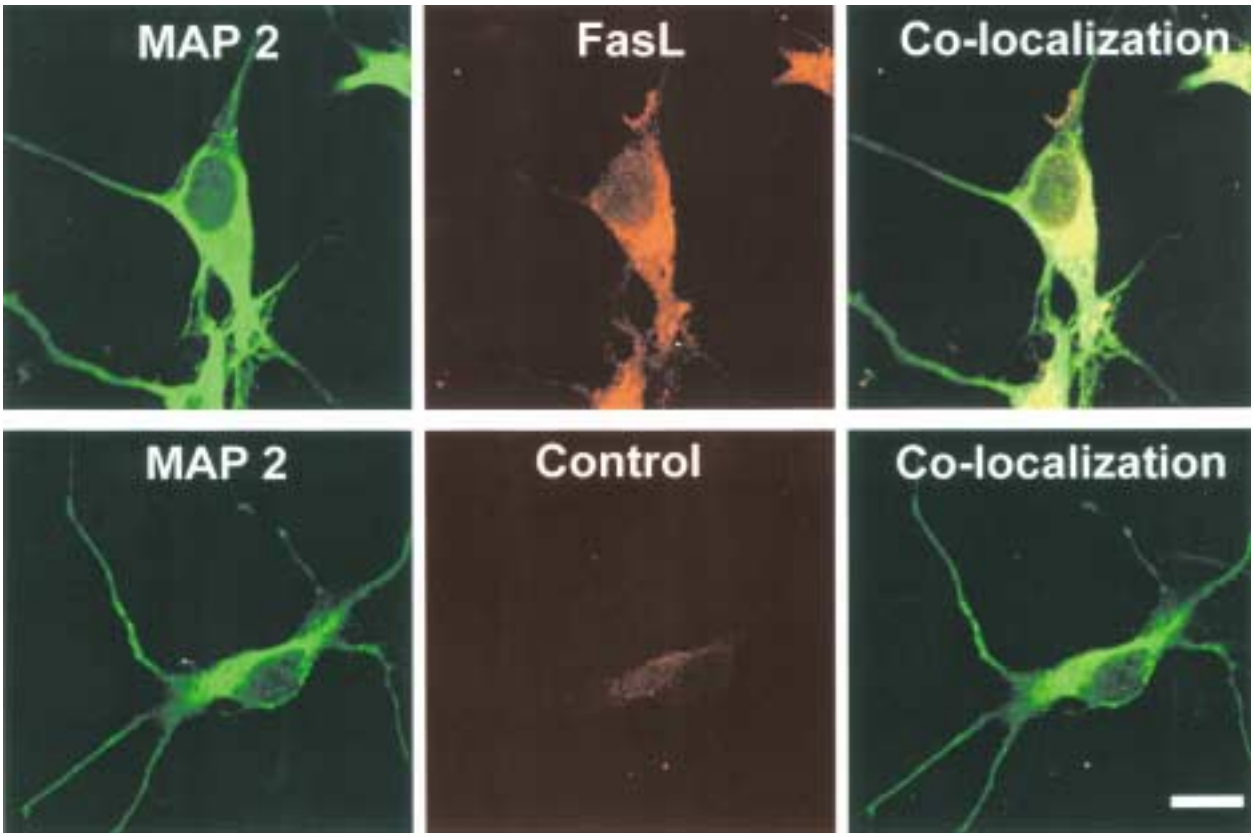


Figure 5. Immunodetection of FasL in cultured neurons. Labeling for FasL in neurons by hamster monoclonal antibody directed against FasL. Neurons were identified by immunostaining for the neuronal cytoskeletal protein MAP2. Primary hamster isotype control antibody was used as a negative control. Scale bar: 10 μm.

according to the manufacturer's protocol (Boehringer Mannheim) using 250 ng of the respective PCR product as a template. For *in situ* hybridization, 20 μm dried cryostat sections were incubated with 2×10^6 cpm of the ^{35}S-UTP (Amersham) labeled probe. After washing, sections were dehydrated in ethanol containing 0.3 M ammonium acetate, air-dried, dipped in film emulsion (NBT-2, Kodak), and exposed at 4°C for 14 days. They were developed in Kodak D19 developer and fixed in 24% sodium thiosulfate. Sections were counterstained with cresyl violet, dehydrated and mounted in DePeX (Gurr).

Hippocampal neuronal cultures. Hippocampal cell cultures were prepared from embryonic Lewis rats as previously described (39). In brief, hippocampi were isolated from embryonic day 17 rats (E17) and the meninges were removed. The brain tissue was dissociated by trituration through a fire-polished Pasteur pipette. Cells were plated onto culture dishes that had been pretreated with poly-L-ornithine (0.5 mg/ml; Sigma) in 0.15 M boric acid followed by laminin (10 μg/ml, gift from A. Ries, MPI, Biochemistry). Dissociated neurons were cultured for 10 to 14 days in Basal Medium Eagle (BME, Gibco, BRL) with the following supplements: 1% glucose (Sigma, 45%), 2% B27-supplement (Gibco, 50x) and 1% fetal calf serum (FCS; Pan System). Thereafter, T lymphocytes were co-cultured with the neurons for 24 hours in the above mentioned medium.

Astrocyte cultures. Astrocytes were prepared from newborn Lewis rats by dissecting hippocampi, removing the meninges and dissociating the tissue through trituration using a fire-polished Pasteur pipette. Cells were suspended and cultured in minimal essential medium with D-valine and 10% FCS. Cells were cultured for approximately two weeks to obtain a confluent monolayer of astrocytes. In order to remove microglia, the culture flasks were agitated on a rotary shaker at 750 rpm for 3 hours and the non-adherent cells removed. Remaining adherent astrocytes were sub-cultured after

trypsinization with trypsin-EDTA and were used for T-lymphocyte co-culture experiments under the same culture conditions (chemically defined medium) as described for the neuronal cells.

Immunohistochemistry and membrane labeling of cultured cells. Cells were washed with phosphate buffered saline (PBS), fixed in 4% PFA, then incubated with primary hamster monoclonal antibody directed against FasL (10 μg/ml, Pharmingen) and a secondary fluorochrome Cy3-conjugated goat anti-hamster antibody (10 μg/ml; Dianova). Following extensive washing, cells were then incubated with a neuron-specific mouse monoclonal antibody recognizing MAP2 (10 μg/ml; Sigma) and secondary DTAF-conjugated goat anti-mouse immunoglobulin (10 μg/ml; Dianova). Optical sections along the z-axis were scanned with a confocal laser-scanning microscope (Leica Inc., Deerfield, IL) using a 63X oil objective. The level of immunocytochemical background labeling was determined in parallel experiments employing an isotype-matched monoclonal hamster antibody (10 μg/ml; Pharmingen) and a secondary fluorochrome Cy3-conjugated goat antibody directed against hamster immunoglobulin.

Live T lymphocyte-neuron interactions were analyzed in the presence of FM1-43 (10 μM), using confocal laser scanning microscopy. The amphipathic fluorescent dye FM1-43 is a well established agent to visualize membrane trafficking (7). During confocal imaging, cultures were maintained at 37°C. Serial confocal images were taken every 5-10 minutes after establishment of T lymphocyte-neuron contact.

Blockage of FasL in T-cell/neuron and T-cell/astrocyte cultures and cytotoxicity assay. For the *in vitro* analysis of the effects of FasL on encephalitogenic $CD4^+$ GFP^+ T lymphocytes, hippocampal neurons or astrocytes (1×10^4/well, culture conditions as described above), respectively, were co-cultured with T lymphocytes (2×10^4 and 5×10^3 T cells/well) in 96 cell plates. Cytotoxicity was assayed using cytofluorometry as described (17). Briefly, the number of target GFP^+ T lymphocytes was evaluated after 24 hours of co-cultivation in relation to a known number of phycoerythrin-labeled plastic beads (Becton-Dickinson), which had been added to the samples before cytofluorometric three color-mode analysis. Propidium iodide positive dead T cells were excluded from the analysis. The neutralizing anti-FasL antibody (clone MFL4) and its isotype control (A19-3) were obtained from Pharmingen and used at a concentration of 40 μg/ml. At least 10 independent measurements were performed per group. Statistical significance was tested using One Way Analysis of Variance (ANOVA) and Student's t-test (SigmaStat 2.03, SPSS Inc.).

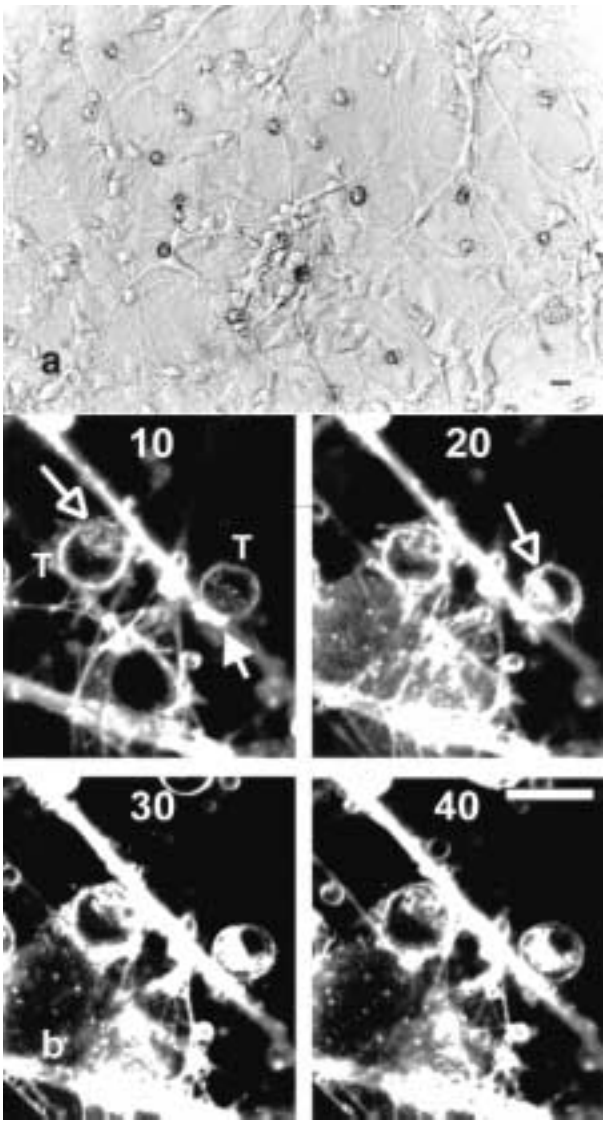


Figure 6. a. Co-culture of CD4 positive T lymphocytes with neurons. Phase contrast image taken after 24 hours. Scale bar: 10 μm. **b.** Cell membrane activity of T lymphocytes and neurites following the formation of stable aggregates as visualized by the amphipathic fluorescent dye, FM1-43. T lymphocytes attach to neurons and neurites as early as 10 min (10) after addition and form stable aggregates, e.g., after 20 min (20). T lymphocytes and neurites show a rapid increase in plasma membrane activity at the points of membrane contact (filled arrow). Distribution of the dye within T cells is polarized toward the site of neurite contact (open arrow). Scale bar: 10 μm.

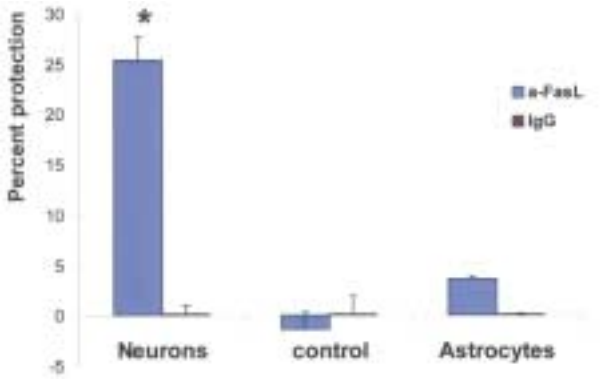


Figure 7. Protective effect of neutralizing hamster anti-FasL monoclonal antibody (a-FasL) on the survival of T_{MBP} GFP positive cells co-cultured with either hippocampal neurons or astrocytes, or cultured alone. Isotype matched hamster immunoglobulin (IgG) was used as control. Following 24 h of co-culture, survival of encephalitogenic T cells was significantly increased by the blockage of neuronal FasL ($p<0.001$). In contrast, no significant effect of anti-FasL antibody on T cell survival could be detected in pure T cell cultures or cultures of T cells with astrocytes. Data are representative of three independent experiments; each data value represents the mean and S.D. of at least ten analyzed samples.

Results

GFP-transduced encephalitogenic T lymphocytes infiltrating the lesioned facial nucleus rapidly undergo apoptosis. Myelin basic protein (MBP)-specific, $CD4^+$ T lymphocyte blasts (T_{MBP}) retrovirally transduced with the GFP gene and transferred to Lewis rats reproducibly induced typical experimental autoimmune encephalomyelitis (EAE) (16). However, when injected immediately after facial nerve transection, the encephalitogenic fluorescent T_{MBP}GFP cells preferentially infiltrated the axotomized facial nucleus (Figure 1a, b). The kinetics of T_{MBP}GFP cell infiltration after i.p. T-cell injection and axotomy are shown in Figure 1c indicating rapid invasion by the T_{MBP}GFP cells beginning on day three and reaching its peak only one day later. Subsequent clearance of the cells was remarkably fast: within a single day (day five) more than 80% of the transgenic cells initially present had disappeared from the CNS parenchyma (Figure 1c). In order to clarify whether encephalitogenic T cells in the lesioned facial nerve nucleus undergo apoptosis, we performed TUNEL labeling. We detected high numbers of TUNEL-positive cells in the lesioned facial nucleus coinciding with T cell infiltration (Figure 2a, b). At the ultrastructural level, apoptotic cells were found to be exclusively T lymphocytes (Figure 2c). It may be important to note at this point that the rat facial nerve paradigm has been studied extensively in the past by our as well as by other groups; apoptosis of neurons, microglia or of any other resident cell type has not been observed under regeneration or retrograde degeneration conditions.

GFP-transduced encephalitogenic T lymphocytes come into close contact with neuronal somata and neuronal cell processes. T_{MBP}GFP cells infiltrating the lesioned facial nerve nucleus were often found in the immediate vicinity of neurons (Figures 1, 2). Electron microscopic analysis revealed that the majority of infiltrating T cells were in direct contact with neuronal surface membranes, and some lymphocytes were even detected inside motoneuron perikarya (Figure 2c). Yet, at the intercellular interface, the surface membranes of both neurons and T cells were always preserved. All T cells in direct physical contact with neurons were found to undergo apoptosis as depicted in Figure 2. In contrast to T lymphocytes, neurons did not show nuclear and cytoplasmic changes characteristic of apoptosis or any other form of cell death. Accordingly, we did not observe a reduction in the number of nerve cells in the lesioned compared to the contralateral facial nucleus (data not shown).

Facial motoneurons express FasL, infiltrating T lymphocytes express Fas. Immunocytochemical analysis of infiltrating T lymphocytes revealed the presence of Fas antigen on both T_{MBP}GFP and recruited T cells (Figure 3a). This finding was confirmed on the ultrastructural level (Figure 3b). At the same time, FasL protein and its messenger RNA were upregulated in the facial nucleus (Figure 4). Immunolabeling for FasL was accentuated at the interface between inflammatory cells and neurons (Figure 4). Both FasL immunoreactivity and FasL *in situ*-hybridization signal could be clearly attributed to motoneurons identified by their distinctive morphology and size (Figure 4). There was no evidence of FasL expression by glial cells.

Cell death of $CD4^+$ T lymphocytes induced by neurons in vitro can be partially blocked by anti-FasL antibody. In order to clarify the functional significance of neuronal FasL in the induction of T-cell death, hippocampal neurons and T_{MBP}GFP cells were co-cultured *in vitro*. Neurons in these cultures form a dense network as shown previously (39) and expressed FasL as shown by immunocytochemical co-localization of FasL with MAP2 (Figure 5). Round T cells were often found adhering to neurons and their neurites (Figure 6a). In

order to study the interaction of $CD4^+$ T cells with neurons in greater detail, adherence of T cells to neurons was monitored at 10 minute intervals using confocal laser microscopy. Neurons and T cells were labeled with the amphipathic fluorescent membrane probe FM1-43. The T lymphocytes in the cultures rapidly attached to the neurons and their cell processes forming stable aggregates during the whole observation period (Figure 6b). Interestingly, attachment of T cells to neurons was followed by an increase in membrane labeling of the neurites and T cells with FM1-43, suggesting elevated membrane trafficking, which was most pronounced at the point of membrane contact and followed by a polarized intracellular uptake of the dye into T cells (Figure 6b).

To test the effect of neuronal FasL on T-cell survival, neutralizing anti-FasL antibody was added to either pure cultures of T_{MBP}GFP cells or co-cultures of T_{MBP}GFP cells with freshly isolated hippocampal neurons or astrocytes, respectively. Cytotoxicity was determined by counting the absolute number of surviving T_{MBP}GFP cells after a culture period of 24 h using a recently developed cytofluorometric cytotoxicity assay (17). Thereby, absolute numbers of living GFP-labelled T cells which survived beyond the co-culture period were determined in relation to a defined sample of PE-fluorescent beads that had been added to the cultures immediately before the analysis (M&M). In neuronal co-cultures, addition of anti-FasL antibody resulted in a statistically significant increase in the number of surviving T cells (25%$\pm$2.2%, $p< 0.001$) when compared to isotypic control antibody. In contrast, no inhibitory effect of anti-FasL on the induction of T cell death could be detected in co-cultures of T cells with astrocytes (3.7%$\pm$0.2%), or in pure T cell cultures (-1.4%$\pm$1.7%) (Figure 7).

Discussion

This study provides strong evidence that neurons participate in the regulation of inflammatory responses in the CNS. Specifically, our results indicate that Fas+ encephalitogenic T cells interact directly with FasL expressing neurons and, upon close cell-cell contact, undergo apoptosis. Thus, an active role for neuronal FasL in the down-regulation of inflammatory processes in the nervous system can be envisioned.

The model of passive transfer EAE induction in combination with facial nerve axotomy allows to study in detail immune-nervous system interactions at the cellular level and in an experimentally well-defined and anatomically circumscribed CNS area. Following facial nerve axotomy alone, there is no detectable invasion of blood-derived cells in this rat model of neuronal regeneration (23). However, it has been shown that induction of active or passive transfer EAE in axotomized animals leads to a preferential accumulation of inflammatory infiltrates in the axotomized facial nucleus (30, 32). Using a recently established method, we have transferred the gene for GFP into MBP-specific encephalitogenic, $CD4^+$ T lymphocytes (16). Now, injected encephalitogenic T cells can be distinguished unequivocally from host-derived recruited cells. We found that high numbers of T_{MBP}GFP cells infiltrated the lesioned facial nerve nucleus. Within the nucleus, the cells came in contact with regenerating motoneurons and underwent apoptosis. Entry of lymphocytes into nerve cells without ensuing nerve cell death has been previously observed *in vitro* (26).

tEAE in Lewis rats manifests itself as an acute monophasic disease with weight loss and ascending paralysis. The recovery phase is generally attributed to apoptosis of infiltrated T lymphocytes (3, 8, 41, 48). However, the cell, or cells, which mediate the induction of T cell death remain to be defined. Apoptotic T lymphocytes have been detected in contact with different resident CNS cells (29, 40, 58). As shown here, contact of T_{MBP}GFP cells with neurons can lead to massive T-cell death.

The immune status of the CNS is conditional. In the healthy brain, inflammation is suppressed (57). However, migration studies have shown that the CNS is under constant lymphocyte surveillance (44, 57). Microglia as well as astrocytes have been found to possess the capacity to present CNS antigen to lymphocytes (18, 19). Moreover, MHC molecules can be induced on CNS cells, including nerve cells (39). Thus, there appears to be a need for a mechanism which helps to protect postmitotic nerve cells against potentially harmful T-cell infiltration.

Expression of FasL has been demonstrated in immune-privileged organs such as the eye and testes. A similar immune-privilege has been proposed for the CNS (21, 36, 50, 52). Indeed, FasL expression in the brain first described by French et al. in neurons (21), has been confirmed by other groups (4, 8, 11, 12, 36, 45, 46, 56). FasL/Fas expression also occurs in multiple sclerosis lesions (8, 11, 12) but its importance in the regulation of CNS immune processes is controversial (2, 10, 46, 47, 56). While the role of FasL in autoimmune disease has been questioned by some groups (2), the authors of a recent study in mice, which lack functional Fas (lpr) or FasL (gld), ascribe a protective role to FasL expression in EAE (47). Our results clearly support the latter view.

Fas expression by T_{MBP}GFP cells as well as by other, secondarily recruited immune cells could provide one mechanism underlying "unspecific T-cell death," i.e., apoptosis of T cells occuring independent of their T-cell receptor antigen specificity (3).

The possibility that binding of neuronal FasL to Fas on T lymphocytes represents an important protective mechanism which is called into action before irreversible damage is inflicted upon the neuron is supported by our *in vitro* analysis. The data confirm cellular cross-talk between neurons and T cells and, at the same time, demonstrate that these interactions can be mediated by FasL and its surface receptor, Fas: FasL-neutralizing antibodies showed a significant protective effect on T-cell survival.

Finally, taking the capacity of lymphocytes to produce neurotrophins into account (13, 28), the possibility should be considered that neurons may actually benefit from T-cell contact under certain conditions. The MBP-specific T lymphocytes employed in this study express significant amounts of both NGF and BDNF (not shown). Thus, close interaction of T lymphocytes with neuronal cells involving vesicle exchange between them could result in trophic support for lesioned neurons during a defined period of time (35).

In conclusion, our results support the view that neurons function as active participants in the regulation of CNS inflammation. This view is in agreement with the finding that nerve cells are able to produce certain cytokines as well as MHC molecules (20, 53). By expressing FasL, neurons are well equipped to actively kill encephalitogenic T cells and thereby limit autoimmune damage. Thus, FasL expression by neurons may constitute a pivotal mechanism contributing to the immune privilege of the CNS.

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